EL 501 637 013 US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Soff *et al.*

Application No.: 08/991,761

Filed: December 16, 1997

For: METHODS AND COMPOSITIONS FOR GENERATING ANGIOSTATIN

Group Art Unit: 1642

Examiner: Minh-Tam Davis

Attorney Docket No.: 10561-004

DECLARATION OF GERALD A. SOFF, M.D., UNDER 37 CFR § 1.132

Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Sir:

GERALD A. SOFF M.D. declares and states that:

1. I am a citizen of the United States, residing at 303 Apple Tree Lane, Wilmette, IL 60091.

2. I presently hold the position of Associate Professor of Medicine at Northwestern University Medical School, Chicago, IL, which position I have held since 2000. From 1991 to 2000, I held the position of Assistant Professor of Medicine, Northwestern University Medical School, Chicago, IL. I have held the positions of: Instructor in Medicine, Beth Israel Hospital, Harvard Medical School, Boston, MA; Visiting Scientist, Massachusetts Institute of Technology, Cambridge, MA; and Fullbright Scholar, Council of International Exchange of Scholars.

3. I received a B.A. from Johns Hopkins University in 1977 and an M.D. from Johns Hopkins School of Medicine in 1981. I completed a three-year internship/residency in medicine at the Medical College of Virginia and a three-year fellowship in

hematology/oncology at Beth Israel Hospital, Harvard Medical School, Boston, MA. I am a Diplomat of The American Board of Internal Medicine, Subspecialty in Hematology.

4. My academic and technical experience, honors, and a list of my publications are set forth in my *curriculum vitae*, attached hereto as Exhibit A.

5. I am a coinventor of present U.S. patent application Serial No. 08/991,761, filed 12/16/97, in the name of Soff *et al.* and entitled "Methods and Composition For Generating Angiostatin." I am familiar with the Office Action dated August 5, 2000 issued therein.

6. In the Office Action dated August 5, 2000 the Examiner rejected claims 59-80, stating that the specification, while being enabling for a method of treating a tumor comprising administering to an animal suffering from such a disease a sulfhydryl donor and urokinase-type plasminogen activator, does not reasonably provide enablement for a method of treating an angiogenic disease comprising administering to an animal suffering from such a disease the following compounds: 1) plasmin and a sulfhydryl donor, wherein the sulfhydryl donor causes the conversion of plasmin to angiostatin *in vivo* or 2) a plasminogen activator and a sulfhydryl donor wherein the sulfhydryl donor converts plasmin to angiostatin, *in vivo*, and the plasminogen activator, urokinase converts plasminogen to plasmin *in vivo* or 3) plasminogen, a plasminogen activator and a sulfhydryl donor, wherein the sulfhydryl donor converts plasmin to angiostatin and the plasminogen activator urokinase, converts plasminogen to plasmin *in vivo*. *See* Office Action at page 2.

7. The Examiner contends that one of skill in the art could not extrapolate from *in vitro* experiments to *in vivo* results and that no working examples or evidence has been provided which would allow one of skill in the art to predict the efficacy of the claimed role of a sulfhydryl donor in producing angiostatin *in vivo* with a reasonable expectation of success.

8. The following experiments have been performed. The results demonstrate the successful use of the methods of the present invention in the treatment of four human cancer patients with a combination of a sulfhydryl donor and two types of plasminogen activators.

Treatment of Human Cancer Patients

9. Four human cancer patients were treated with a combination of a sulfhydryl donor and a plasminogen activator. These angiostatic combinations are referred to as the "Angiostatic Cocktail." Captopril was used as the sulfhydryl donor. Urokinase (uPA) or tissue plasminogen activator (tPA) was used as the plasminogen activator. The compounds administered and their doses and schedules of administration are summarized in Exhibit B, Table 1. In addition, one of the patients (Case #2) received a single treatment with uPA alone (*i.e.*, without the captopril).

10. The term angiostatin, here refers to the naturally occurring human angiostatin isoform, which includes the first four and approximately 85% of the fifth kringle of plasminogen (*see* specification page 14, lines 13-24 and Figure 16).

11. For the detection and quantification of angiostatin, whole blood was collected and platelet-poor plasma prepared by centrifugation (2500g at 4°C, for 15 minutes). The plasma was then diluted 1:40 Tris-Glycine running buffer, and electrophoresed under non-reducing conditions on 12% polyacrylamide gels (NOVEX, San Diego, CA) in Tris-Glycine running buffer. The polyacrylamide gels are electrotransferred to a polyvinylene difluoride (PVDF) membrane (Immobilon, Millipore, Bedford, MA) per standard technology. The membrane was then blocked for >30 minutes in blocking buffer (1% bovine serum albumin in Tris-buffered saline) and probed with a conjugate of GMA086 (Green Mountain Antibodies, Vermont) and alkaline phosphatase (Sigma) (Green Mountain Antibodies, Vermont). GMA086 has been shown to bind to the kringle domains of plasminogen and angiostatin. Blots were developed using 5-bromo-4-chloro-3-indoyl-phosphate/nitroblue tetrazolium (Kirkegaard & Perry Laboratories, Gaithersburg, MD). Direct conjugation of the primary antibody avoids the artifact of a second antibody (i.e. rabbit antimouse IgG-alkaline phosphatase) which could cross-react with the human IgG in the plasma samples.

12. For the estimate of angiostatin concentration, a standard curve of a range of angiostatin concentrations (10-700 ng/ml) was resolved alongside the test samples. The concentration of angiostatin was determined by scanning densitometry of the Western blots with background subtracted. The density of each standard protein was plotted against its

concentration and regression analysis was used to calculate the concentration of angiostatin present in plasma and ascites. Each blot evaluated contained a standard curve ($r^2 > 0.9$).

13. Blood samples, in sodium citrate anticoagulant, were taken before, and at various times after, treatment was begun. Platelet-poor plasma was isolated from the blood, and stored frozen until tested.

14. Western blots were performed on the plasma samples (diluted 1:20) in Tris-Buffered saline (20 nM Tris, pH 8.0, 150 nM NaCl) as described in Example 1 (specification, page 37). The results of the Western blots performed on plasma samples taken from Case #2 showed an increase in angiostatin levels. As noted above, this patient received uPA alone and also in combination with captopril. Unexpectedly, the increase in angiostatin levels was observed as a result of either treatment. This indicates that captopril, or other exogenous sulfhydryl donors, may not be necessary to convert plasmin to angiostatin in some *in vivo* situations. Such situations may include the presence of mesothelioma or other cancers that can release endogenous sulfhydryl donors or that, in some other way, mediate conversion of plasmin to angiostatin.

15. The generation of angiostatin antigen in plasma of patients receiving the Angiostatic Cocktail was evaluated by Western blot. In order to detect angiostatin antigen, platelet poor plasma was prepared from the pre-treatment specimens, from 90 minutes into the 6 hour continuous infusions, and at completion of the 6 hour continuous infusion. The plasma was assayed by Western blot for the presence of the angiostatin antigen and a modified, semi-quantitative Western blot assay was used to quantitate the angiostatin antigen. Western blots were performed on plasma from cases #2 and #3, both of whom had received an Angiostatic Cocktail. For Case #3, (tPA-based Angiostatic Cocktail), angiostatin antigen was undetectable in pre-treatment plasma, but was detectable 90 minutes into the treatment and at completion of the 6 hour continuous infusion of the Angiostatic Cocktail. Similar results were observed for 5 consecutive days. Case #2 received a urokinase-based Angiostatic Cocktail. This patient also demonstrated the absence of angiostatin antigen in pre-treatment plasma but showed detectable angiostatin antigen at completion of the Angiostatic Cocktail treatment.

16. The results from the assays of plasma from Cases #2 and #3 are set forth in Exhibit C, Figures 1 and 2. Figure 1 illustrates Case #3 (tPA-based Angiostatic Cocktail with captopril), for the first three of five successive days. Figure 2 illustrates Case #3 for the fourth and fifth of the five successive days. Figure 2 also illustrates Case #2 who received a urokinase-based Angiostatic Cocktail with captopril. For Case #3 (tPA-based Angiostatic Cocktail with captopril), on five successive days, angiostatin was undetectable in pre-treatment plasma, but was detectable 90 minutes into the treatment and at completion of the 6 hour continuous infusion. Case #2 (urokinase-based Angiostatic Cocktail with captopril) also demonstrated no angiostatin in pre-treatment plasma and detectable angiostatin at completion of the Angiostatic Cocktail. Thus, the results demonstrate that the angiostatin antigen is readily detectable during and immediately after the 6 hour infusion of the Angiostatic Cocktail. In addition, the results show that both the urokinase and tPA-based Angiostatic Cocktail increased angiostatin levels.

17. To determine if the Angiostatic Cocktail was indeed inducing antiangiogenic activity as well as angiostatin antigen, plasma samples from the patients were tested for antiangiogenic activity. The lysine-binding protein fraction from pre-treatment and post-treatment plasma was affinity purified by lysine-Sepharose chromatography, this fraction would include angiostatin, plasminogen, and plasmin:alpha-2-macroglobulin and plasmin:alpha-2-antiplasmin complexes. If the Angiostatic Cocktail was inducing angiostatin, the lysine-binding protein fraction would be expected to possess increased antiangiogenic activity, as plasminogen does not possess antiangiogenic activity. Exhibit D, Figure 3 is a bar graph showing the antiangiogenic activity induced by the Angiostatic Cocktail. A 1:200 dilution of the lysine-binding protein fractions were added to endothelial cell media. At 72 hours, there was a suppression of the endothelial cell proliferation by the post-treatment samples (solid bars) compared with pre-treatment samples (stippled bars). Exhibit D, Figure 3 shows that the lysine-binding fraction of plasma exhibited an increase in inhibitory activity on endothelial cell proliferation, consistent with the predicted induction of angiostatin and anti-angiogenic activity. This effect was noted both for case #2, receiving a uPA based cocktail, and for case #4, receiving a tPA based cocktail.

18. The antiangiogenic activity induced by the administration of a plasminogen activator alone was also evaluated. To do this, the lysine-binding proteins were purified from platelet-poor plasma samples obtained after the administration of uPA alone or uPA plus captopril to Case #2. First, 1 ml of platelet-poor plasma was diluted 1:40 with 20 mM Tris, pH 8.0, and incubated with 8 ml of Lysine-Sepharose (pre-equilibrated in 20 mM Tris, pH 8.0) for >12 hours with gentle shaking at 4°C. (In preliminary experiments, the 8:1 ratio of resin:plasma bound >99% of angiostatin and plasma). The plasma supernatant was separated by gentle centrifugation (2000 x g), and the resin was washed 3 times with 40 ml of 100 mM NaCl, 20 mM Tris, 5 mM EDTA, pH 8.0. After washing and repeat centrifugation, the bound proteins were eluted with 4 ml of 200 mM EACA, 20 mM Tris, 5 mM EDTA, pH 8.0, and the elution dialyzed to 40 mM NaCl, 20 mM Hepes, 5 mM EDTA, pH 8.0, and spin concentrated (MW cutoff = 10,000) to 500 microliters and tested in endothelial cell proliferation assays. On Western blot and Coomasie-stained polyacrylamide gel electrophoresis, the lysine-binding fractions contained plasminogen, angiostatin, and plasmin:alpha-2-macroglobulin and plasmin:alpha-2-antiplasmin complexes.

19. To evaluate the antiangiogenic activity induced by a plasminogen activator alone, the lysine-binding fractions of the plasma samples taken from Case #2 when she had received uPA alone were analyzed in a cellular proliferation assay as follows. Bovine aortic endothelial cells were plated in 24-well culture dishes at 1.0 x 10^4 cells/well in DMEM supplemented with 2.5% heat-inactivated calf serum, 100 Units/ml penicillin G, 100 mg/ml streptomycin, and the cells were incubated overnight at 37°C in a humidified incubator. On each of the following three days, fresh medium containing 3 ng/ml human bFGF (R&D Systems, Minneapolis, MN) alone or with various amounts of the lysine-binding fractions was added. As a positive control, 100 nM affinity-purified, cell-free angiostatin, produced as described in Example 6 (specification at page 54) was used. After 72 hours of treatment, cells were washed with phosphate buffered saline, dispersed with trypsin-EDTA, and the cell number was determined by counting from duplicate wells using a Coulter counter. The results are shown in Exhibit E, Figure 4. This exhibit is a bar graph of cell numbers versus various amounts of the lysine-binding fractions of platelet-poor plasma samples taken from Case #2, Cycle 1, day 1. As shown in Figure 4 the administration of urokinase (uPA) alone

induced antiangiogenic activity as measured by this endothelial cell proliferation assay. These data indicate that antiangiogenic activity may be induced by plasminogen activators alone, although not necessarily as potently as by the plasminogen activators and free sulfhydryl donors in combination.

20. Case #1 was a 14 year old girl with recurrent Ewing's sarcoma of the left pelvis. After she could not tolerate chemotherapy or radiation therapy due to her extensive prior treatment, she received multiple cycles of the combination of captopril and urokinase. The doses and schedules of administration are summarized in Exhibit B, Table 1. For the first several months, she received the captopril-urokinase combination for 3 consecutive days every 2 weeks. Subsequently, she received the combination 2 consecutive days every 3 weeks for a total of one year. A "cycle" refers to the 2 or 3 days of treatment, plus the days off treatment, until the therapy was begun again 2 or 3 weeks later. The western blots revealed generation of angiostatin-related protein, which included free angiostatin as well as complexes of plasminogen-related antigen. Routinely, in the plasma samples from after the administration of the Angiostatic Cocktail an approximately 150 kD band was observed on Western blots, which is believed to be a plasmin:alpha-2-antiplasmin complex. This is based on the ability to affinity purify the protein complex with lysine-Sepharose, the binding of the complex to 3 different anti-plasminogen kringle antibodies (GMA 086, and GMA 039 from Green Mountain Antibodies, Burlington, VT, and VAP from Frank Castellino, Notre Dame, IN), and also the binding of the protein band to a monoclonal antibody to alpha-2-antiplasmin (American Diagnostica).

A complex of approximately >250 kD was also observed after treatment with the Angiostatic Cocktail. This is believed to be a plasmin:alpha-2-macroglobulin complex. This is based on the ability to affinity purify the large protein complex with lysine-Sepharose, the binding of the complex to 3 different anti-plasminogen kringle antibodies (GMA 086, and GMA 039 from Green Mountain Antibodies, Burlington, VT, and VAP from Frank Castellino, Notre Dame, IN), and the binding of the protein band to a goat anti-human alpha-2 macroglobulin antibody (Affinity Biologicals, Ontario, Canada).

21. After three months of treatment with the Angiostatic Cocktail, the patient achieved a complete remission. At this point a pelvic CT scan indicated the tumor had

undergone liquefaction. The tumor was reduced to necrotic material, and was interpreted as an abscess on a repeat CT scan and no solid tumor was evident at this point. The necrotic material was drained percutaneously and another CT scan indicated no remaining evidence of disease. The patient continued to receive the Angiostatic Cocktail on a maintenance basis, and she experienced no side-effects except for the mild, transient hypotension during the period of time she was receiving the captopril. She continued to be treated with the Angiostatic Cocktail for a full year. At that point, since the United States Food and Drug Administration had mandated that urokinase be withdrawn from the market, the angiostatic therapy was terminated. She remained in complete remission, off therapy for more than one year when her cancer relapsed.

22. The biological effects of *in vivo* treatment with the angiostatic combination of a sulfhydryl donor (captopril) in combination with a plasminogen activator such as Urokinase (uPA) or tissue plasminogen activator (tPA) was evaluated in a total of four patients. All of these patients, their treatment and the results of their treatment are summarized in Exhibit B, Table 1. As Table 1 indicates, Case #2 received the uPA-based Angiostatic Cocktail but Cases #3 and #4 received a tPA-based cocktail, as uPA was, by then, no longer available. The data in Table 1 shows that, following the teaching of the present specification, Angiostatic Cocktails comprised of two different types of plasminogen activators, uPA or tPA combined with the sulfhydryl donor captopril can *in vivo* produce marked tumor regression to complete remission in human patients with a variety of tumor types.

23. In view of the foregoing, I conclude and it is my opinion that others skilled in the art would also conclude that the present specification provides enablement for methods of treating an angiogenic disease comprising administering to an animal suffering from such a disease a plasminogen activator and a sulfhydryl donor, wherein the sulfhydryl donor converts plasmin to angiostatin, *in vivo*, and the plasminogen activator converts plasminogen to plasmin *in vivo*.

24. Furthermore, I conclude and it is my opinion that others skilled in the art would also conclude that the present specification provides enablement for methods of treating an angiogenic disease comprising administering to an animal suffering from such a disease a plasminogen activator alone, wherein the plasminogen activator converts plasminogen to

plasmin *in vivo*.

25. I declare further that all statements made in this Declaration of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Respectfully submitted,

Date 2/13/2001

Gerald Soff

Gerald A. Soff, M.D.

Attachments:

Exhibit A: *Curriculum Vitae* of Gerald A. Soff, M.D.
Exhibit B: Table 1
Exhibit C: Figures 1 & 2
Exhibit D: Figure 3
Exhibit E: Figure 4

EXHIBIT A

CURRICULUM VITAE **Gerald A. Soff, M.D.**

Address: 303 Apple Tree Lane
Wilmette, IL 60091

Date of Birth: October 12, 1955 Social Security No.: 058 44 4104

Place of Birth: New York, New York

Education:

1977 B.A. Cornell University (1973-1975) and Johns Hopkins University (1975-1977)
1981 M.D. Johns Hopkins School of Medicine

Postdoctoral Training:

Internship/Residency:
1981-1984 Medicine, Medical College of Virginia
Fellowship:
1985-1988 Hematology/Oncology, Beth Israel Hospital, Harvard Medical School, Boston, MA.

Licensure and Certification:

1982	Virginia Medical License #0101034414
1986	Massachusetts Medical License #56474
1991	Illinois State Medical License #036-083173
DEA #	BS 0748333

Board Certification:

1984	Diplomat of The American Board of Internal Medicine.
1988	Diplomat of The American Board of Internal Medicine, Subspecialty in Hematology.

Academic Appointments/Previous Positions:

1984-1985	Fulbright Scholar, Council for International Exchange of Scholars.
1988-1991	Instructor in Medicine, Beth Israel Hospital, Harvard Medical School, Boston, MA.
1986-1991	Visiting Scientist, Massachusetts Institute of Technology, Cambridge, MA.
1991-2000	Assistant Professor of Medicine, Northwestern University Medical School, Chicago, IL.

Academic Appointments/Current Position:

2000-2001 Associate Professor of Medicine, Northwestern University Medical School, Chicago, IL.

Invited Lectures (Past 2 years):

1. "Angiogenesis-A Potential Target For Cancer Therapy." American College of Physicians/American Society of Internal Medicine, Illinois Chapter Scientific Meeting. October 23, 1998.

2. "Angiostatin 4.5, A Naturally Occurring Angiogenesis Inhibitor." Inauguration of Walther Cancer Center, Notre Dame University, December 17, 1998.

3. "Angiogenesis, From Theory To Practice." Medical Grand Rounds at Michael Reese Hospital, Chicago, IL., August 12, 1999.

4. "Angiogenesis, and Antiangiogenic Therapy." Plenary Presentation for the Annual Meeting of The American College of Veterinary Pathologists, November 16, 1999.

5. "Thrombocytopenia In The Adult Medical Patient; Diagnosis and Management," Medical Grand Rounds, Highland Park Hospital, January 11, 2000.

Awards and Honors:

1978-1979 Henry Strong Denison Scholarship recipient of the Johns Hopkins University School of Medicine.

1984-1985 Fulbright Scholar, Council for International Exchange of Scholars.

Major Committee Assignments/Hospital:

1998 Medical Director, Anticoagulation Dosing Service, Northwestern Memorial Hospital

1998 Member, Pharmacy And Therapeutics Committee, Northwestern Memorial Hospital

1996 Medical Director of "Rube Walker" Blood Center, at Northwestern Memorial Hospital

1995-1996 Assistant Director of Rube Walker Blood Center, at Northwestern Memorial Hospital

Major Committee Assignments/ Medical School:

1991-1997 Member of Northwestern University Intramural Grant Committee

1996-1997 Chairman, Northwestern University Intramural Grant Committee

Membership In Professional Organizations:

1993 Member of American Society of Hematology.

1997 American Association For Cancer Research

1997 Central Society For Clinical Research

Major Research Interests:

1) Angiogenesis
2) Thrombosis and Hemostasis
3) Vascular biology

Mentoring Research Trainees:

Mentored Medicine Residents In Research Electives

1993 Serena Yoon M.D.; First Prize in Residents Research Symposium
1995 Lisa Boggio M.D.; First Prize in Residents Research Symposium.
1997 Suzie Chi M.D.; First Prize in Residents Research Symposium.
1997-1998 Douglas Tomasian M.D.; First Prize in Residents Research Symposium.
1998 Anne Mellott M.D.;
1998 Philip Simonian M.D.

Mentored Hematology Oncology Fellows In Research Programs

1994-1995 Elaine Lee Wade M.D.
1995-1997 Przemyslaw Twardowski M.D.
1997- 1999 Lisa Boggio M.D.
1997- 1999 Jerome Hong M.D.

Mentored Hematology Oncology Faculty In Research Programs

1993-1995 Judith Senderowicz M.D.
1993-1995 Ann Traynor M.D.

Mentored Post-Doctoral Research Fellows

1994-1998 Stephen Gately Ph.D.

Mentored Graduate Students

1999-present Hao Wang

Principal Clinical and Hospital Service Responsibilities:

Attend on benign Hematology consult service, 4 months per year.
Medical Director of:

1) Anticoagulation Dosing Service, Northwestern Memorial Hospital
2) The Blood Center, Northwestern Memorial Hospital

BIBLIOGRAPHY

Original Reports:

1. **Soff GA**, Levin J. Familial multiple coagulation factor deficiencies. I. Review of the literature: Differentiation of single hereditary disorders associated with multiple factor deficiencies from coincidental concurrence of single factor deficiency states. *Semin Thromb Hemostas*. 7:112-148, 1981.

2. **Soff GA**, Levin J, Bell WR. Familial multiple coagulation factor deficiencies. II. Combined

factor VIII, IX, and XI deficiency and combined factor IX and XI deficiency: Two previously uncharacterized familial multiple factor deficiency syndromes. *Semin Thromb Hemostas*. 7:149-169, 1981.

3. **Soff GA**, Sica DA, Marlar RA, Evans HJ, Qureshi GD. Protein C levels in nephrotic syndrome. Use of a new enzyme-linked immunoadsorbent assay for protein C antigen. *Am J Hemat*. 22:43-49, 1986.

4. Margulis T, David M, Maor N, **Soff GA**, Grenadier E, Palant A, Aghai E. The von Willebrand factor in myocardial infarction and unstable angina: a kinetic study. *Thromb Haemost.* 55:366-368, 1986.

5. **Soff GA,** Kadin ME. Tocainide-induced reversible agranulocytosis and anemia. *Arch Intern Med.* 147:598-599, 1987.

6. Weiss P, **Soff GA,** Halkin H, Seligsohn U. Decline of proteins C and S and factors II, VII, IX, and X during the initiation of warfarin therapy. *Thrombosis Res.* 45:783-790, 1987.

7. Jackman RW, Beeler DL, Fritze L, **Soff GA,** Rosenberg RD. The human thrombomodulin gene is intron depleted: Nucleic acid sequences of the cDNA and gene predict protein structure and sites of regulatory control. *Proc Nat Acad Sci. (USA)* 84:6425-6429, 1987.

8. **Soff GA**, Levin J. Thrombocytopenia associated with repletion of iron in iron-deficiency anemia. *Am J Med Sci* 295:35-39, 1988.

9. **Soff GA**, Jackman RW, Rosenberg RD. Expression of thrombomodulin by smooth muscle cells in culture. (Different effects of tumor necrosis factor and cyclic AMP on thrombomodulin expression by endothelial cells and smooth muscle cells in culture). *Blood* 77(3):515-518,1991.

10. Weiler-Guettler H, Yu K, **Soff G**, Gudas LJ, Rosenberg RD. Thrombomodulin gene regulation by cAMP and retinoic acid in F9 embryonal carcinoma cells. *Proc Nat Acad Sci. (USA)* 89:2155-2159, 1992.

11. **Soff GA,** Green D. Autoantibody to von Willebrand factor in systemic lupus erythematosus. *J. Lab. Clin. Med.,* 121:424-430, 1993.

12. Cornwell TL, **Soff GA,** Traynor AE, Lincoln TM. Regulation of the expression of cyclic GMP-dependent protein kinase by cell density in vascular smooth muscle cells. *J. Vasc. Res.* 31:330-337, 1994.

13. Traynor AE, Cundiff D, **Soff GA**. cAMP influence on transcription of thrombomodulin and myogenic proteins in smooth muscle is dependent on de novo synthesis of a protein intermediate. *J. Lab. Clin. Med.* 126:317-323,1995.

14. **Soff GA**, Senderowicz J, Gately, S., Verrusio E, Weiss I, Brem S, Kwaan HC. Expression of Plasminogen Activator Inhibitor Type 1 By Human Prostate Carcinoma Cells Inhibits Primary Tumor Growth, Tumor-Associated Angiogenesis, And Metastasis To Lung And Liver In An Athymic Mouse Model. *J. Clin. Invest.* 96:2593-2600, 1995.

15. Gately S, **Soff GA**, Brem S. The potential role of basic fibroblast growth factor (bFGF) in the transformation of cultured primary human fetal astrocytes and the proliferation of human glioma (U-87) cells. *Neurosurgery* 37:723-732, 1995.

16. Gately S, Twardowski P, Stack MS, Patrick M, Boggio L, Cundiff DL, Schnaper HW, Madison L, Volpert O, Bouck N, Enghild J, Kwaan HC, **Soff GA**. Human prostate carcinoma cells express enzymatic activity that converts human plasminogen to the angiogenesis inhibitor, angiostatin. *Cancer Research* 56:4887-4890, 1996.

17. Green D, Maliekel K, Sushko E, Akhtar R, **Soff GA**. Activated-protein-C resistance in cancer patients. *Haemostasis* 27:112-118, 1997.

18. Gately S, Twardowski P, Stack MS, Cundiff DL, Grella D, Castellino FJ, Enghild J, Kwaan HC, Lee F, Kramer RA, **Soff GA**. The Mechanism of Cancer-Mediated Conversion of Plasminogen to the Angiogenesis Inhibitor, Angiostatin. *Proc Nat Acad Sci. (USA)*, 94:10868-10872, 1997.

19. **Soff GA**, Cornwell TL, Cundiff DL, Wade EL, Lincoln TM. Smooth Muscle Cell Expression Of Type I Cyclic GMP-Dependent Protein Kinase Is Suppressed By Continuous Exposure To Nitrovasodilators, Theophylline, cyclic GMP, and cyclic AMP. *J Clin Invest.* 100:2580-2587, 1997.

20. Lannutti BJ, Gately ST, Quevedo ME, **Soff GA**, Paller AS. Human angiostatin inhibits murine hemangioendothelioma tumor growth *in vivo*. *Cancer Research* 57:5277-5280, 1997.

21. Mauceri HJ, Hanna NN, Beckett MA, Staba M-J, Gorski DH, Bigelow K, Heinmann R, Gately S, **Soff GA**, Kufe DW, Weichselbaum RR. Angiostatin and Radiation Target the Tumor Vasculature. *Nature* 394:287-291, 1998.

22. Lucas R, Holmgren L, Garcia I, Jimenez B, Mandriota SJ, Borlat F, Sim BK, Wu Z, Grau GE, Shing Y, **Soff GA**, Bouck N, Pepper MS. Multiple forms of angiostatin induce apoptosis in endothelial cells. *Blood* 92: 4730-41, 1998.

23. Gorski DH, Mauceri HJ, Salloum RM, Gately S, Hellman S, Beckett MA, Sukhatme VP, **Soff GA**, Kufe DW, Weichselbaum RR. Potentiation of the antitumor effect of ionizing radiation by brief concomitant exposures to angiostatin. *Cancer Research* 58: 5686-5689, 1998.

24. Lincoln TM, Dey NB, Boerth NJ, Cornwell TL, **Soff GA**. Nitric oxide--cyclic GMP pathway

regulates vascular smooth muscle cell phenotypic modulation: implications in vascular diseases. *Acta Physiol Scand* 164: 507-15, 1998.

25. Stack MS, Gately S, Bafetti LM, Enghild JJ, **Soff GA**. Angiostatin inhibits endothelial and melanoma cellular invasion by blocking matrix-enhanced plasminogen activation. *Biochem J.* 340: 77-84, 1999.

26. Gately S, Hong J, Fishman D, Kleinman M, Kaplan E, Zagzag D, Schultz R, Cundiff D, Park S, Enghild JJ, Stack MS, **Soff GA**. Angiostatin4.5: A naturally occurring human angiogenesis inhibitor. *Cancer Research* (Submitted).

27. Gohongi T, Fukumura D, Boucher Y, **Soff GA**, Todoroki T, Jain RK. Suppression of angiogenesis at a second site by an orthotopic gallbladder tumor: Role of TGF-1. *Nature Medicine* 5:1203-1208, 1999.

28. Gradishar WJ, **Soff G**, Liv J, Cisneros A, French S, et al. A pilot trial of suramin in metastatic breast cancer to assess antiangiogenic activity in individual patients. *Oncology* 58: 324-333, 2000.

<u>Chapters In Textbooks</u>

1. **Soff GA** and Rosenberg RD. Physiology of Coagulation: The Fluid Phase. <u>In</u> Hematology of Infancy and Childhood, DG Nathan and FA Oski (eds.), W.B. Saunders Co., 1993, pp.1534-1560.

2. **Soff GA**. Disseminated Intravascular Coagulation. <u>IN</u> Conn's Current Therapy, RE Rakel (ed.), W.B. Saunders Co., 1993, pp.378-379.

3. **Soff GA**. Thrombotic Thrombocytopenic Purpura. <u>IN</u> Conn's Current Therapy, RE Rakel (ed.), W.B. Saunders Co., 1993, pp.379-381.

4. **Soff GA**. Acquired von Willebrand's Disease. <u>IN</u> Anticoagulants: Physiologic, Pathologic, and Pharmacologic, D Green (ed.), CRC Press, 1994, pp. 157-168.

5. **Soff GA**. Coagulation Mechanisms: Hemostasis and Thrombosis. <u>IN</u> Physiologic and Pharmacologic Bases of Anesthesia, VJ. Collins (ed.), Williams & Wilkins, 1996, pp.214-232.

<u>Invited Reviews:</u>

1. Kwaan HC, **Soff G**. The plasminogen-plasmin system in malignant tumors. *Journal of the Robert H. Lurie Cancer Center of Northwestern University*. 5:15-24, 1996.

2. Kwaan HC, **Soff G**. Management of Thrombotic Thrombocytopenic purpura and Hemolytic Uremic Syndrome. *Seminars in Hematology* 34:159, 1997.

3. **Soff, GA**. Angiostatin and angiostatin-related proteins. *Cancer and Metastases Reviews* 19:97-107, 2000.

Abstracts:

A1. **Soff GA**, Levin J, Bell WR. Familial multiple coagulation factor deficiencies. Clin Res. 29:864A, 1981.

A2. **Soff GA**. Thrombocytopenia resulting from repletion of iron in iron deficiency anemia. Virginia Medical Monthly 110:116, 1983.

A3. **Soff GA**, Marlar RA, Sica D, Qureshi GD, Evans HJ. Protein C and Protein C inhibitor levels in the nephrotic syndrome. Clin Res. 31:875A, 1983.

A4. **Soff GA**, Weiss P, Halkin H, Griffin JH, Seligsohn U. Half-life of human protein S antigen as determined by a new enzyme-linked immunoadsorbent assay. Blood 66 suppl. 1:353a, 1985.

A5. Beeler D, Fritze L, **Soff G**, Jackman R, Rosenberg R. Human thrombomodulin cDNA: Sequence and translated structure. Thromb Haemostas. 320:1168, 1987.

A6. **Soff GA**, Jackman RW, Rosenberg RD. Expression of thrombomodulin by smooth muscle cells in culture. Blood 74 supple. 1:136a, 1989.

A7. **Soff GA**, Green D. Autoantibody to von Willebrand factor in systemic lupus erythematosus. Clinical Research. 40:425A, 1992.

A8. Oh D, Kwaan HC, Goolsby C, Verrusio E, Yoshida E, **Soff G**. Urokinase is mitogenic on prostate tumor cell lines. Blood 80 supple. 1:1754a, 1992.

A9. **Soff GA**, Kwaan HC, Verrusio E, Yoshida, E, Oh DY. Modulation of tumor growth and angiogenesis by PAI-1. Clinical Research 43:697a, 1993.

A9 **Soff GA**, Cundiff DL, Senderowicz J, Cornwell, TL, Traynor, AE. Regulation of The Expression of Cyclic GMP-Dependent Kinase In Vascular Smooth Muscle Cells. Blood 82 supple 1:279a, 1993.

A10. Traynor AE, Senderowicz J, Cundiff DL, **Soff GA**. Mechanism of cyclic amp stimulation of thrombomodulin expression In Vascular Smooth Muscle Cells. Blood 82 supple 1:279a, 1993.

A11. Gately S, **Soff GA**, Takano S, Klagsburn M, Brem S. The transforming property of signal peptide-basic fibroblast growth factor (FGF-2) in human astrocytes. Proc. Am. Assoc. Canc. Res. 35: 42, 1994.

A12. Gately S, **Soff GA**, Brem S. Quantitation of basic fibroblast growth factor (bFGF) in human brain tumor tissues by ELISA. Proc. Am. Assoc. Canc. Res. 36:24, 1995.

A13. Gately S, Takano S, **Soff GA**, Brem S. Human glioma cell invasion *in vitro* is correlated with

the release of urokinase-type plasminogen activator (u-PA), and decreased expression of cell-associated basic fibroblast growth factor (bFGF). Proc. Am. Assoc. Canc. Res. 36:98, 1995.

A14. Zipfel G, Gately S, **Soff GA**, Engelhard H, Kwaan H, Brem, S. D-penicillamine induces the expression of plasminogen activator inhibitor-1 (PAI-1) mRNA and protein in human glioma cells at clinically relevant concentrations. Proc. Am. Assoc. Canc. Res. 36:589, 1995.

A15. **Soff GA**, Senderowicz J, Takano S., Verrusio E., Weiss I., Brem S., Kwaan HC. PAI-1 expression by human prostate carcinoma cells inhibits primary tumor growth, tumor-associated angiogenesis and metastases in an athymic mouse carcinoma model. Proc. Am. Assoc. Canc. Res. 35: 101, 1995.

A16. **Soff GA**, Gately ST, Twardowski PW, Stack S, Kwaan HC. Identification and characterization of Plasminogen-Angiostatin Converting Enzyme from human prostate carcinoma cells. Blood 86 supple. 1: 742a, 1995.

A17. **Soff GA**, Gately S, Twardowski P, Stack MS, Boggio LN, Bacallao, R, Kwaan HC. Characterization of Plasminogen-Angiostatin Converting Enzyme from human prostate carcinoma cells. Proc. Am. Assoc. Canc. Res. 37:59, 1996.

A18. **Soff GA**, Twardowski P, Stack MS, Kwaan HC, Gately S. Cancer cell-derived mechanism for the generation of human angiostatin. Proc. Am. Assoc. Canc. Res. 38:#1775, 1997.

A19. Gately S, Twardowski P, Stack MS, Cundiff DL, Grella D, Castellino FJ, Enghild J, Kwaan HC, Lee F, Kramer RA, **Soff GA**. The mechanism of cancer-mediated conversion of plasminogen to the angiogenesis inhibitor angiostatin. Fibrinolysis & Proteolysis 11 (Supple. 3) 39, 1997.

A20. Hanna NN, Mauceri HJ, **Soff GA**, Gately S, Beckett MA, Weichselbaum RR. Effect of angiostatin on the growth and radiation response of human cancer xenografts. Proceedings of the American Society of Surgical Oncology. 1997.

A21. Gorski D, Mauceri H, Hanna N, Gately S, **Soff G**, Weichslebaum R. Radiation and angiostatin statistically inhibit the growth of Lewis lung carcinoma. Proceedings of the American Society of Surgical Oncology. 1997.

A22. **Soff GA**, Hong J, Fishman D, Kleinman M, Kaplan E, Zagzag D, Schultz R, Cundiff D*, Park S, Enghild J, Stack MS, Gately S, Angiostatin4.5: A Naturally occurring human angiogenesis inhibitor. Blood 92 (Supple. 1) 174a, 1998.

A23. **Soff GA**, Hong J, Fishman D, Schultz R, Cundiff D, Park S, Enghild J, Stack MS, Gately S. Angiostatin4.5: A Naturally occurring human angiogenesis inhibitor. Proc. Am. Assoc. Canc. Res. 40: #4088, 1999.

A24. **Soff GA**, Hoppin EC, Cundiff D, Schultz R, Kunz P. Therapeutic Application of an Angiostatic Cocktail For Patients With Refractory Cancer. Proc. Am. Assoc. Canc. Res. 41: #1960, 2000.

A25. Wang H, Hong J, Boggio L, Cundiff D, **Soff GA**. Surface dependence of Plasminogen Conversion To Angiostatin4.5. Proc. Am. Assoc. Canc. Res. 41: #3101, 2000.

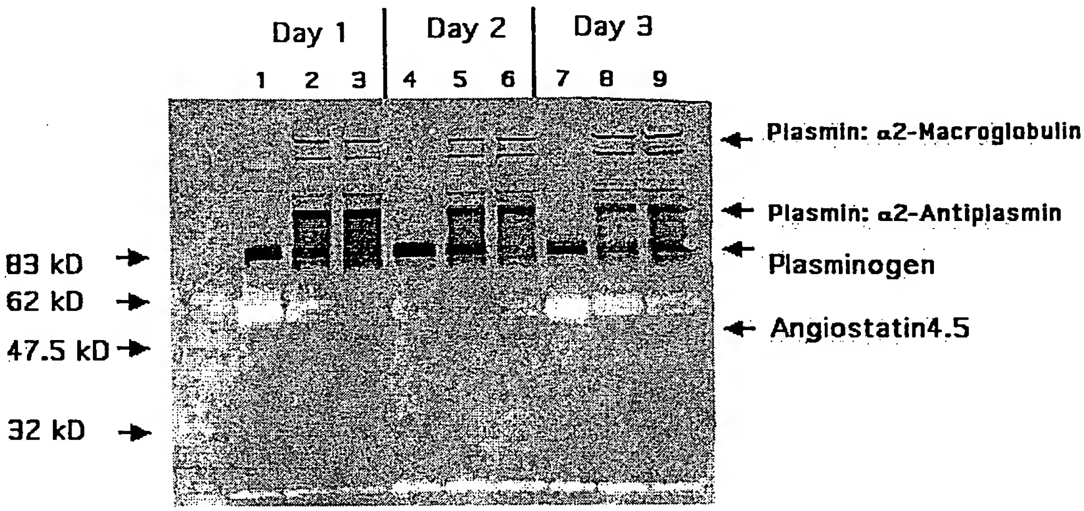
EXHIBIT B

Table 1. Summary of Cases Treated With An Angiostatic Cocktail

Case Number	1	2	3	4
	14 year old girl	61 year old woman	39 year old woman	50 year old Man
Diagnosis	Ewing's sarcoma	Mesothelioma	Colon Cancer	Pancreatic Cancer
Prior Therapy	Vincristine/ cyclophosphamide, adriamycin, ifosfamide, etopaside) Local radiation (54 Gy)	Adriamycin	CPT-11	Other experimental Agents
Angiostatic Cocktail Regimen	uPA/Captopril	uPA/Captopril	tPA/Captopril	tPA/Captopril
	uPA; 500 U/kg bolus, then 200 U/kg/hr X 6 hours Captopril 0.3 mg/kg at 0 hr and 4 hr. Treatment daily X 3 days every two weeks	uPA; 500 U/kg bolus, then 200 U/kg/hr X 6 hours Captopril 0.3 mg/kg at 0 hr and 4 hr. Treatment daily X 3 days every two weeks	tPA 7 mg bolus, then 3 mg/hr X 6 hours Captopril 20 mg at 0 hr. and 4 hr. Treatment daily X 5 days, every two weeks.	tPA 5 mg/hr X 6 hours Captopril 25 mg at 0 hr. and 4 hr. Treatment daily X 5 days, every two weeks.
Increase In Angiostatin Levels.		From <10 to 40 nM	From <10 to 80 nM	From <10 to 200 nM
Clinical Response to Angiostatic Cocktail	Liquefaction (Complete remission)	Partial Response (80% reduction in tumor size) in combination with Gemcytabine/ cisplatinum	N/A (Patient only treated for 3 weeks)	N/A (Patient only treated for 3 weeks)

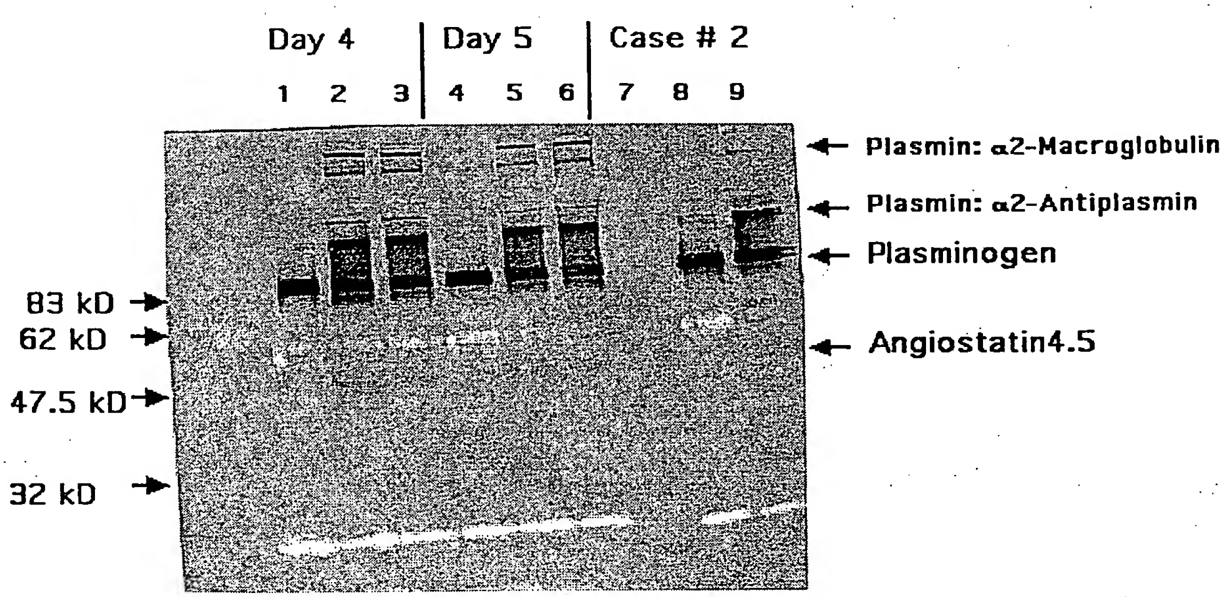
EXHIBIT C

Figure 1



Lanes 1, 4, 7; Case #3, Pretreatment from each day
Lanes 2, 5, 8; Case #3, 90 minutes into treatment
Lanes 3, 6, 9; Case #3, Completion of 6 hour continuous infusion

Figure 2



Lanes 1, 4; Case #3, Pretreatment from each day
Lanes 2, 5; Case #3, 90 minutes into treatment
Lanes 3, 6; Case #3, Completion of 6 hour continuous infusion

Lane 8; Case #2, Pretreatment, Day #1
Lane 9; Case #2, Completion of 6 hour continuous infusion, Day #1

EXHIBIT D

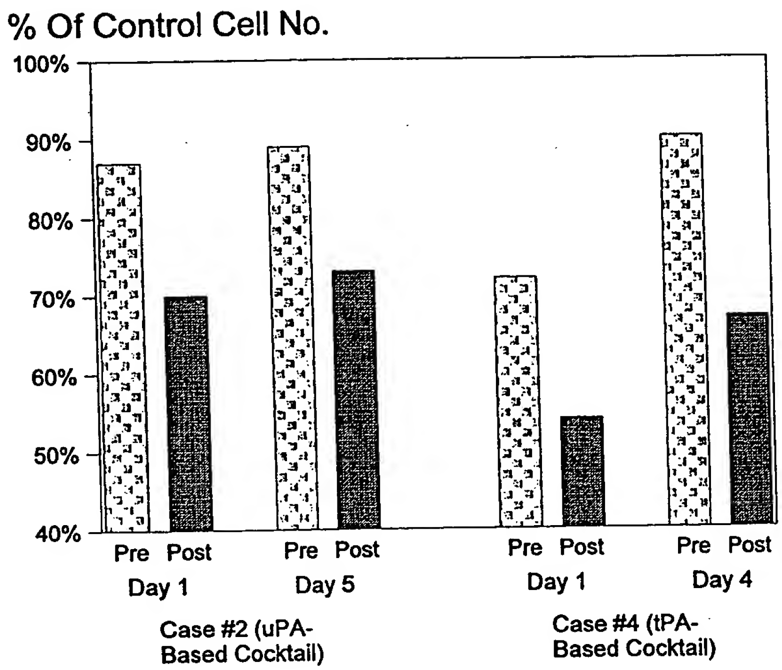

% Of Control Cell No.
100%
90%
80%
70%
60%
50%
40%
Pre Post
Day 1
Pre Post
Day 5
Case #2 (uPA-Based Cocktail)
Pre Post
Day 1
Pre Post
Day 4
Case #4 (tPA-Based Cocktail)

Figure 3

EXHIBIT E

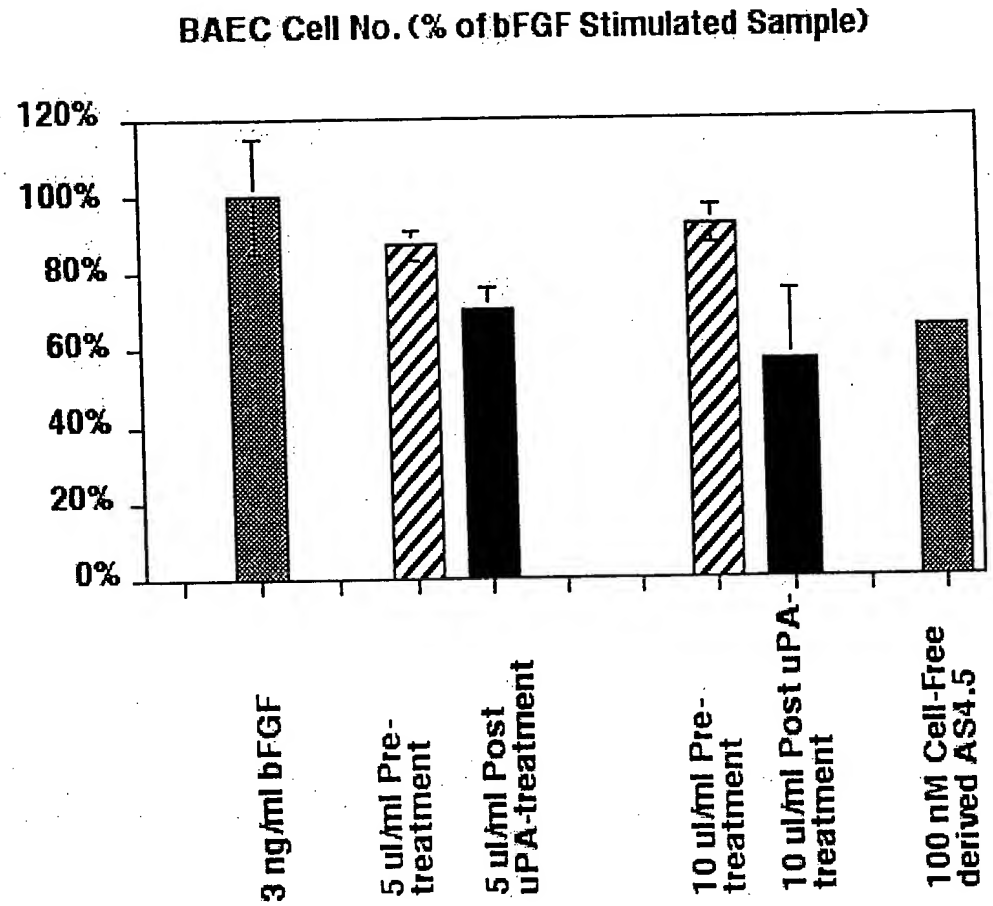

BAEC Cell No. (% of bFGF Stimulated Sample)
120%
100%
80%
60%
40%
20%
0%
3 ng/ml bFGF
5 ul/ml Pre-treatment
5 ul/ml Post uPA-treatment
10 ul/ml Pre-treatment
10 ul/ml Post uPA-treatment
100 nM Cell-Free derived AS4.5

Figure 4